Constitutive Antiviral Immunity at the Expense of Autoimmunity

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In this issue of *Immunity*, Funabiki et al. (2014) have identified in mice a mutation of the *IFIH1* gene, encoding the viral receptor MDA5 that causes constitutive IFN production and fatal autoimmune disease. The authors show that the autoimmune disease-associated variant of human MDA5 is permanently switched on.

Two major fields of research, autoimmune disease and virus infection, converged in 2006 when the interferon (IFN)-induced helicase 1 gene, *IFIH1*, encoding a major pathogen-recognition receptor, MDA5, for viral infection (Yoneyama et al., 2004), was associated in one of the first genome-wide association studies (GWAS) with risk of the autoimmune disease type 1 diabetes (T1D) (Smyth et al., 2006). Binding of double-stranded RNA (dsRNA) to MDA5 triggers the signaling molecules IPS-1 (or MAVS), IRF3, and IRF7, causing transcription of the antiviral type I IFN (Ivashkiv and Donlin, 2013). This IFN, which can originate from hematopoietic (dendritic cells and macrophages) (Funabiki et al., 2014; Ivashkiv and Donlin, 2013) and nonhematopoietic cell lineage (referenced in Funabiki et al., 2014) tissues, induces the transcription of hundreds of IFN-inducible genes to contain the infection. MDA5 function in antiviral responses is so important that several viruses have evolved gene functions to decrease MDA5 activity (Ivashkiv and Donlin, 2013).

In T1D, the *IFIH1* risk allele of a nonsynonymous SNP (rs1990760) encodes Thr at position 946 in the C-terminal RIG-1-like domain of MDA5, whereas the nonpredisposing or disease-protective allele encodes Ala946, which for this SNP is the ancestral or "wild-type" (WT) allele (Smyth et al., 2006; Molineros et al., 2013) Depending on which side of the coin you look at, Thr946 provides a 16% increase in T1D risk in 65% of the British population or Ala946 causes 16% reduced risk or protection in 35% of the population. Thr946/Thr946 homozygous individuals, which account for 42% of the population, are at 35% increased risk of T1D (compared to non-Thr946 or Ala946-positive carriers) (Smyth et al., 2006).

Deep resequencing of the *IFIH1* gene from hundreds of T1D patients and controls found low-frequency variants, which probably result in loss of function, and were protective for T1D; that is, normal or increased function of IFHI was predisposing to T1D (Nejentsev et al., 2009). Furthermore, it was shown that the genetic haplotype or chromosome bearing the Thr946 allele produced more IFIH1 mRNA than the Ala946 haplotype, suggesting that the Thr946 allele has a gain-of-function effect in its production of type 1 IFN and/or sensitivity to the effects of IFN (Downes et al., 2010; Molineros et al., 2013; Robinson et al., 2011). Subsequent genetic studies in other immune diseases found the same association in Crohn's disease, ulcerative colitis, and psoriasis at genome-wide significance (immunobase.org), and by taking a number of studies into account, the same variant and additional polymorphisms in *IFIH1* are also, with near certainty, associated with susceptibility to systemic lupus erythematosus (SLE) (Molineros et al., 2013; Ivashkiv and Donlin, 2013).

Using the now well-established approach of generating and identifying chemically-induced mutations in mice and screening for interesting immune- and disease-associated phenotypes, Funabiki et al. (2014) identified a mutation in the IFIH1 gene, encoding Gly821Ser, in the Helicase C-terminal domain of MDA5. They show convincingly that Ser821 causes an SLE-like phenotype in the mutant mice: nephritis or inflammation of the kidney, characterized by lymphocyte infiltration with immunoglobulin and complement deposition and high levels of interleukin-6 (IL-6), IFN-β, and tumor necrosis factor-α (TNF-α). There are widespread systemic inflammation effects, including upregulation of IL-6 and IFN-β in other organs such as heart and lung. In the spleen, increased numbers of effector $CD4^+$ and $CD8^+$ effector T cells, activated dendritic cells and macrophages (producing IL-6), and plasma B cells were observed, along with serum anti-nuclear and anti-ds DNA autoantibodies and hyperimmunoglobulinemia (Figure 1). Nephritis was observed as early as 6 weeks after birth, and 60% of the mice died within 24 weeks. Note that these were heterozygous mice, containing only one copy of the mutant Ser821 allele: Ser821/Ser821 homozygous mice developed even more severe inflammation and rarely survived for 4 weeks after birth. Disease was completely dependent on the function of IPS-1, a mitochondrial membrane protein to which MDA5 binds to trigger the IFN signaling cascade.

Remarkably, while this mutation knocks out the ATPase activity of MDA5, making the receptor unresponsive to RNA ligands, the mutant molecule is constitutively active in its function as an inducer of IFN production owing to a conformational rearrangement. Very reassuringly, in 2009 an independent group had reported mutations (generated and analyzed in vitro) in the helicase domain of human MDA5, for example positions 327 and 826, (Figure 1) that also caused constitutive activation of the molecule and continuous, RNA-independent, type 1 IFN production (referenced in Funabiki et al., 2014).

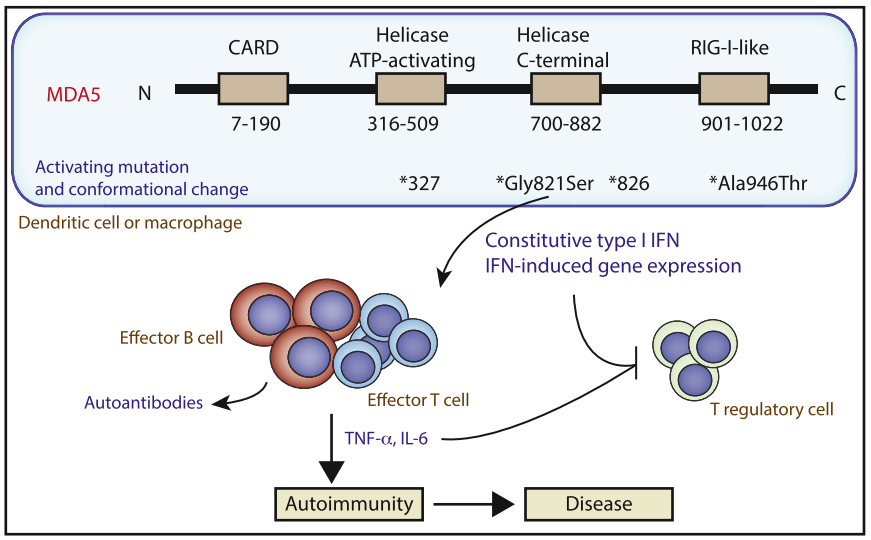

Figure 1. Gain-of-Function Mutations of MDA5 Cause Hyperproduction of Type 1 Interferons and Autoimmunity

Variants of *Ifih1* in mice (Gly821Ser) or *IFIH1* in human (Ala946Thr) and other variants generated in previous studies (positions 327 and 826) alter the conformation of this viral RNA recognition intracellular receptor to make it function constitutively in its role as an inducer of the type 1 IFN pathway. This increase in basal IFN causes severe SLE-like autoimmune disease in this new mouse model, and the human allele, Thr946, is a predisposing factor in SLE and other diseases. Chronic IFN production leads to a multitude of immune phenotypes involving many effector and regulatory components of the immune system with numerous negative and positive feedback loops. For example, T regulatory cells function to suppress inflammation and yet type 1 IFN can inactivate these cells and IL-6 inhibits their differentiation. Dendritic cells produce IFN, and this can activate the dendritic cells in their priming of T cells. In humans, carriage of the Thr946/Thr946 genotype is neither necessary nor sufficient for the development of autoimmune disease: for disease to develop, many genes and their predisposing alleles (immunobase.org) are required for acting in a permissive environment.

Most remarkably, Funabiki et al. go on to show that the human allele Thr946 has the same activating conformation and downstream effects, including constitutive IFN production. The side chain of Ala946 has been modeled to make close contact with the helicase domain (Molineros et al., 2013). Because carriage of Thr946 by itself in humans does not cause noticeable lupus-like symptoms, Thr946 does not have as large effects as the chemical Ser821 mutation in their cell assay systems. The present study will prompt the field to look more closely at the immune system in Thr946/Thr946 homozygous individuals versus Ala946/Ala946 subjects for similar effects. Analysis of the effects of Thr946 in vivo in mice engineered for this specific variant could also be informative. A previous study has already shown in SLE patients with anti-ds DNA autoantibodies that the Thr946 allele increases sensitivity to IFN, leading to increased IFN-induced gene expression, including the viral resistance genes, *IFIT1* and *MX1* (Robinson et al., 2011). Serum IFN levels and SLE autoantibodies are closely correlated suggesting a causal relationship, supported by the fact that several other SLE susceptibility genes function in the IFN response, including *TYK2* and *PTPN22* (immunobase.org). Interestingly, this phenotype was only observed in patients with autoantibodies (Robinson et al., 2011), which could be indicative of the role of many genes and their allelic variants acting together to produce an autoimmune-prone genetic background in which the penetrance or effect of Thr946 is more pronounced. Furthermore, the present study immediately poses the following question: do rare, more penetrant (that is, doubling the risk of disease or greater) variants of *IFIH1* exist in the human population that by themselves can cause SLE?

It seems likely that genetically driven, heightened or chronic type I IFN production (or indeed administration of IFN-α in patients), which has been associated not only with SLE but also with Sjogren's syndrome, systemic sclerosis, myositis, rheumatoid arthritis, and most recently, with T1D (Ferreira et al., 2014), predisposes in humans to increased effector B and T cell activation, autoantibodies, and effector cytokines (Ivashkiv and Donlin, 2013). Patients with SLE and with other diseases are often positive for an IFN-induced gene-expression transcriptional signature that can be detected in blood samples (Ivashkiv and Donlin, 2013). A very similar signature is found in infants at high risk of T1D before they develop T1D autoantibodies, and one of the signature genes is *IFIH1* (Ferreira et al., 2014).

Genetic activation of this disease-associated IFN pathway in humans might not on its own be sufficient to drive autoimmunity to overt disease, but in combination with other predisposing allelic variation at an array of genes, for example those that encode negative regulators of the immune system functioning to prevent harmful inflammation and autoimmunity, such as CTLA-4, PTPN22, GP183 (EBI2), and IL-10 (immunobase.org), would make a clinical diagnosis more likely. However, even if newborn children have very high doses of autoimmune disease susceptibility alleles and dysregulated pathways, the penetrance of such a genetic profile is very heavily determined by mostly unknown environmental factors. For example, frequent viral infections in very young children could well be a cofactor, along with an autoimmune genetic profile, in the development of subclinical autoimmunity, which might manifest, again, depending on genes and environment, many years later in clinical diagnosis (Ferreira et al., 2014). At least in mice, it has been reported that commensal bacteria, the microbiota, contribute to the maintenance of basal levels of type 1 IFN production under physiological conditions (Ivashkiv and Donlin, 2013). Disturbance of the assembly of a healthy microbiota early in life, either through host gene variants or environmental factors such as the use of antibiotics, diet, and infections, could well be a factor in immune disease susceptibility and help explain the familial clustering of common disorders (referred to as "missing heritability").

GWAS analyses of common disease have provided major insights into the molecules, cells, and pathways involved in complex, multifactorial disorders. Although in their infancy, these insights

are leading to the identification of therapeutic targets and the potential repositioning of existing drugs with good safety profiles that modulate the genetically-validated pathways active in patients or those at risk of the disease (Plenge et al., 2013). What clues do these new results offers for therapeutic strategies in autoimmune disease? As the authors discuss, targeting the ATPase activity of MDA5 might not be that useful, notwithstanding the reasonable position that pharmacological inhibition of an essential antiviral defense molecule might not be safe. Nevertheless, this study and others indicate that targeting more downstream autoimmune events as a consequence of activating variants of MDA5, such as inhibition of the IL-6 receptor or of TNF-α or depletion of (antigen-specific, if possible) effector B and T cells and/or improving immunosuppressive T regulatory cell functions, such as CTLA-4 or IL-10, might provide safe therapeutic approaches. There are obviously already successful drugs in use clinically for some of these targets (Plenge et al., 2013). Nevertheless, dose and frequency of administration of the appropriate drug in humans (results from mice might not translate) should be determined in relatively small, open-label, statistically-designed, mechanistic studies or trials to investigate immunological efficacy before launching into large and expensive late-phase trials. The beauty of this new single-gene model of autoimmune disease is that it is caused by a single gene that is also an autoimmune disease gene in humans and could therefore be useful to explore therapeutics preclinically. As discussed recently (Plenge et al., 2013), knowledge from human genetics and genomics studies is, and will continue, transforming medicine.

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How T Cells Lose Their Touch

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T cells are among the most sensitive of cells, but in this issue of *Immunity*, Honda et al. (2014) demonstrate that effector T cells must lose their touch within hours to protect the host from immunopathology.

In this issue of *Immunity*, Honda et al. (2014) use a delayed-type hypersensitivity (DTH) model to look at how highly sensitive T cells turn off responses at sites of inflammation. One of the major problems that prevented prior studies from being able to address this issue was asynchrony. The DTH model offered a way to synchronize the response to antigen and observe it through the intact ear skin by two-photon microscopy. Honda et al. (2014) take advantage of the fact that recruitment of activated T cells into sites of inflammation is antigen independent. Therefore, when mice are injected with a strong adjuvant and a mixture of keyhole limpet hemocyanin (KLH) and ovalbumin peptide (OVA) subcutaneously and then challenged by intradermal injection of KLH in the ear pinnae 7 days later, the activation of a few KLH-specific cells in the dermis leads to recruitment of more KLH- and OVA-specific cells over the next couple of days (Honda et al., 2014). The inflammation induced by the T cells leads to ear swelling, and the OVA-specific T cells migrate within the inflamed dermis in search of antigen. Intravenous OVA peptide introduced at this point rapidly permeates into the inflamed site and loads onto I-A^b molecules to activate OVA-specific T cells, leading to arrest of the T cells within 1 min. Thus, the activation process can be synchronized and the kinetics of the effector cell response to antigen in an inflammatory setting could be studied in detail.

Honda et al. (2014) first asked whether the apparent desensitization of the T cells was due to loss of antigen, other changes in the APCs, or a change in the T cells. They found that antigen was still active by